Journal of Natural Products
Vol. 51, No. 2, pp. 212-216, Mar-Apr 1988

NOVEL CLEISTANTHANE DITERPENOIDS FROM *POGOSTEMON AURICULARIS*[1]

FALAK A. HUSSAINI, SUNITA AGARWAL, RAJA ROY, OM PRAKASH, and ABOO SHOEB*

Central Drug Research Institute, Lucknow 226001, India

ABSTRACT.—Three novel diterpenoid acids isolated from the whole plant of *Pogostemon auricularis* have been characterized as cleistanth-13,15-dien-18-oic acid and 7-hydroxy- and 7-acetoxycleistanth-13,15-dien-18-oic acids.

Our continued search for new therapeutic agents from medicinal plants endemic to India (1–3) led to the examination of *Pogostemon auricularis* Hassk (Lamiaceae) whose EtOH extract exhibited spasmolytic activity on preliminary screening. From the bioactive residue, obtained from the hexane-soluble extract of the whole plant, three new diterpenes have been isolated. Their structure elucidation is discussed in the present communication.

The first terpene **1**, designated as auricularic acid, mp 220°, exhibited the presence of an exocyclic methylene group (ν max 3070, 1644, 890 cm^{-1}; δ 4.57, 4.65), a free carboxylic group (ν max 3380, 1680 cm^{-1}), and a vinylic side chain (δ 5.02, 5.04, 6.0). In addition, it contained two methyl groups situated in similar environments and resonating almost at the same frequency (δ 1.22, 0.71) as C-18 and C-20 methyl groups in gummiferolic acid (4). With these functionalities, the molecular formula, $C_{20}H_{30}O_2$, $[M]^+$ at *m/z* 302.2353, suggested **1** to be a tricyclic diterpene. Compound **1** was converted to the corresponding methyl ester **2** (δ 3.48, COO*Me*) with ethereal CH_2N_2.

The dominant feature in the mass spectra of **1** and **2** was the presence of a conspicuous fragment ion at *m/z* 94 arising from the rupture of ring C and, thus, locating the exocyclic methylene and the vinylic groups at any two of C-11, C-12, C-13, or C-14. The decoupling experiments on **1** indicated the vinylic side chain to be situated at a secondary carbon atom that had a methine proton on one side and a quaternary carbon (exomethylene) on the other. For example, irradiation at the frequency of the vinylic methine (δ 6.0) simplified the multiplicities of the vinylic methylene (δ 5.02, 5.04), while the allylic methine (δ 2.82 dd, J=9.2 and 4.6 Hz) was converted into a doublet (J=4.6 Hz). Further, the irradiation at δ 2.82 affected the complexities of a methine (δ 1.50) and those of vinylic methine. Conversely, irradiation at δ 1.50 simplified the multiplicity of the allylic methine to a doublet (J=9.2 Hz).

The conclusion that the exocyclic methylene and the vinylic side chain were vicinal was further reinforced by the ozonolysis of **1**. This afforded an aldehyde **3** in low yield with $[M]^+$ at *m/z* 304, consistent with a composition $C_{19}H_{28}O_3$, and a ketone **4** as the major product, which was characterized by its spectral data and its conversion to the corresponding alcohol **5** and acetate **6**. However, the keto aldehyde **7** was never found as a product of the ozonolysis. Besides steric considerations, the difference in the reactivities of the two sites towards ozone may be attributed partly to the difference in their nucleophilicity; the formation of the ozonide of the exocyclic double bond is favored in comparison to the less substituted vinylic double bond. A base peak at *m/z* 95.0498 $[C_6H_7O]^+$ in the mass spectrum of **4** representing the fragment ion **8** (5) corroborates the vicinal disposition of the above two functionalities and implies the preferred placement of the exocyclic methylene and the vinylic side chain at C-13 and C-14, respectively.

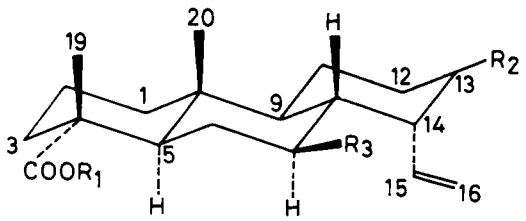
[1]CDRI Communication No. 3750.

Further evidence for the structure **1** for auricularic acid was found by a ^{1}H-nmr study of the aldehyde **3**. Irradiation at δ 9.79 (d, J=9 Hz, *CH*O) converted H-14 (dd, J=9 and 4.5 Hz) into a doublet (J=4.5 Hz).

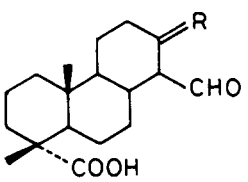
The structure **1** was finally confirmed by the dehydrogenation of auricularic acid over Pd/C, which gave two products. The less polar of the two, mp 108–109°, was identified as 1,7-dimethyl-8-ethylphenanthrene; the other, mp 141°, was characterized as cleistanth-8,11,13-trien-18-oic acid [**9**]. These two new products confirmed the location of the exomethylene and the vinylic groups in **1** at C-13 and C-14, respectively. Compound **1** may thus be formulated as cleistanth-13,15-dien-18-oic acid.

The complete stereostructure of **1** has been determined with the help of 2D ^{1}H-^{1}H (COSY, NOESY) and ^{1}H-^{13}C (Hetero COSY) nmr studies and is reported separately (6).

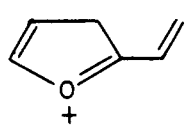
The second terpene **10**, an amorphous solid, $[M]^+$ at *m/z* 318 ($C_{20}H_{30}O_3$), gave an acetate, mp 178°, $[M]^+$ at *m/z* 360, which was identical in all respects to the third terpene **11** isolated as a natural product. Possessing functionalities similar to **1** and in a similar environment, **10** may be considered as a hydroxy derivative of **1**. This view was further reinforced by Jones's oxidation of **10** to yield the ketone **12**, mp 154°, $[M]^+$ at *m/z* 316 ($C_{20}H_{28}O_3$), which under modified Wolff-Kishner reduction conditions (7) gave auricularic acid [**1**] (Scheme 1).



	R_1	R_2	R_3
1	H	$=CH_2$	H
2	Me	$=CH_2$	H
4	H	$=O$	H
5	H	H,OH	H
6	H	H,OAc	H
10	H	$=CH_2$	OH
11	H	$=CH_2$	OAc
13	Me	$=CH_2$	OH
14	Me	$=CH_2$	OAc



3 R=CH_2
7 R=O



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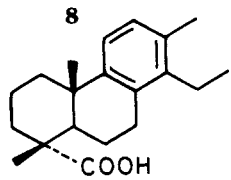

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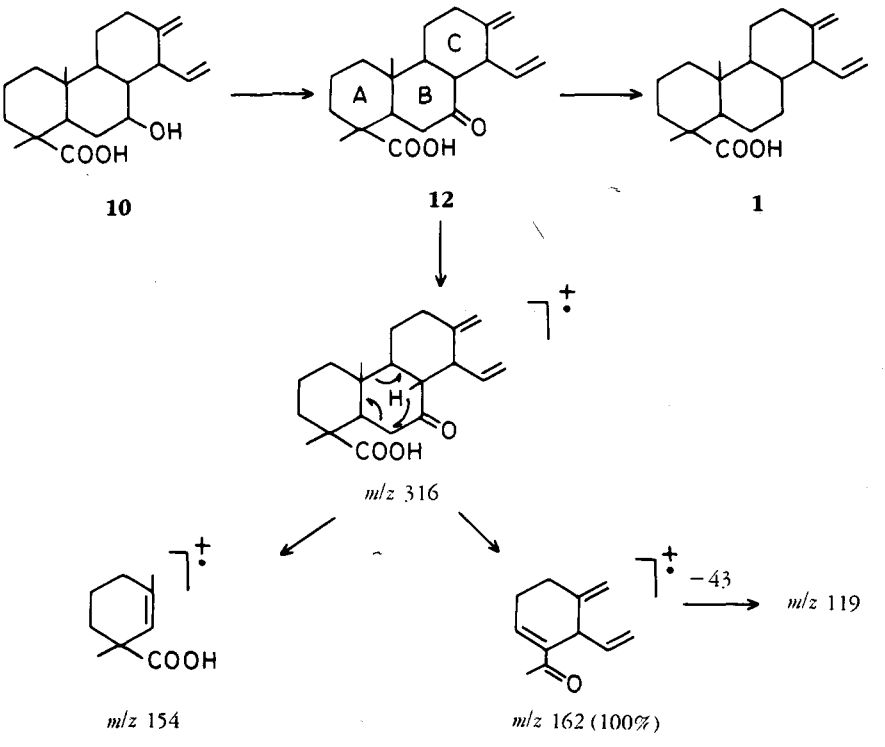

SCHEME 1

The hydroxy group of **10** may be placed on any one of the three rings of **1**. However, its presence in ring C is ruled out on the basis of the study of the mass spectrum of **10** that showed conspicuous fragment ions at *m/z* 94, 146, 164, 193, and 206 arising from the rupture of ring C. This, together with the observed deshielding of H-14 in **10** and **11** and their respective methyl esters **13** and **14** and in **12** compared to **1** suggests the presence of an oxygen function in ring B, probably at C-7. Another piece of evidence in support of this view was provided by the observation of two conspicuous fragment ions at *m/z* 154 and 162 (base peak) in the mass spectrum of **12** resulting from the rupture of ring B in the manner depicted in Scheme 1.

The final proof in favor of structure **10** was achieved by the study of 400 MHz ^{1}H-nmr spectra of **10** and **11**. The H-7 located at δ 4.52 in **11** [ddd, $J_{7,6eq}=4.6$; $J_{7,6ax}=11.0$ and $J_{7,8ax}=12.1$ Hz] was confirmed by specific proton decoupling technique. Thus, for example, irradiation at δ 4.52 caused an enhancement in the C-7 signal resonating at δ 77.0 in ^{13}C-nmr spectrum of **11**. These, together with the broad band (BB) and single frequency off-resonance decoupling methods in their ^{13}C-nmr spectra, permitted the assignment of all the carbon atoms in **10** and **11**. Further, the multiplicity associated with H-7 mentioned above conclusively demonstrated C-7 as the locus of equatorial hydroxy and acetoxy groups in **10** and **11**, respectively.

Compound **11** inhibited 10, 19, and 69% response of spasmogens at 2.5, 5, and 10 μg/ml, respectively, on guinea pig ileum (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. ^{1}H-nmr spectra were recorded on a Perkin-Elmer R-32 instrument (90 MHz) and a Bruker WM 400 instrument (400 MHz).

Mass spectra were obtained on a JEOL D-300 instrument with a JMA-2000 data system. All compounds gave satisfactory elemental analyses.

PLANT MATERIAL.—The whole plant of *P. auricularis* was collected in the month of February from Thalacauvery, Karnatak, India. A voucher specimen has been deposited in the herbarium of the Botany Section of the Institute.

EXTRACTION.—Dried and finely powdered plant material (3 kg) was extracted with hexane (5 × 5 liters). The extract was concentrated in vacuo to leave a residue (25 g) that was separated into acidic and nonacidic fractions by treatment with 1% NaOH solution. The basic solution on acidification followed by extraction with Et_2O gave the acidic fraction (20.2 g). The nonacidic fraction (3.3 g) on cc (Si gel) afforded methyl linolenate and sitosterol. The acidic fraction on similar treatment yielded a mixture of diterpenoid acids (3.0 g) from which **1** (0.75 g), **10** (0.15 g), and **11** (0.27 g) were obtained after rechromatography (Si gel; $AgNO_3$) of the mixture.

Auricularic acid [**1**].—Mp 220° (MeOH); $[\alpha]^{25}D$ + 15.2° (*c* = 2, $CHCl_3$); ir ν max (KBr) 3380, 3070, 2930, 1680, 1644, 1450, 1232, 1226, 918, 890 cm^{-1}; ms *m/z* $[M]^+$ 302.2353, 287, 257, 241, 207, 149, 148, 135, 134, 120, 94, 87, 80.

Methyl ester **2**.—Amorphous solid; ir ν max (KBr) 3450, 2950, 1738, 1458, 1232, 1190, 1165, 1105, 1000, 925, 900, 838, 790 cm^{-1}; ^1H nmr (90 MHz) δ 5.95 (1H, m, H-15), 4.96 (1H, dd, *J* = 10 and 1.0 Hz, H-16), 4.90 (1H, dd, *J* = 18 and 1.0 Hz, H-16'), 4.50, 4.43 (1H each, q, *J* = 2 Hz, 2H-17), 3.48 (3H, s, $COOCH_3$), 2.68 (1H, dd, *J* = 9 and 4.5 Hz, H-14), 1.2 (s, 19-Me), 0.52 (s, 20-Me); ms *m/z* $[M]^+$ 316, 301, 257, 241, 222, 162, 161, 148, 147, 134, 121, 101, 94.

OZONOLYSIS OF **1** TO YIELD **3** AND **4**.—Treatment of a solution of **1** (200 mg) in CH_2Cl_2 (100 ml) at −70° with a current of ozone for 4 h followed by usual work-up afforded **3** and **4** in 5 and 50% yields, respectively.

14-Formylcleistanth-13-en-18-oic acid [**3**].—Mp 170°; ir ν max (KBr) 2975, 1740, 1700, 1460, 1280, 1262, 1182, 1160, 1150, 980, 913, 912 cm^{-1}; ^1H nmr (90 MHz) δ 9.79 (1H, d, *J* = 9 Hz, *CHO*), 4.96 and 4.81 (1H each, q, *J* = 2 Hz, 2H-17), 3.13 (1H, dd, *J* = 9 and 4.5 Hz, H-14), 1.26 (s, 19-Me), 0.73 (s, 20-Me); ms *m/z* $[M]^+$ 304, 208, 276, 275, 260, 208, 194, 150, 135, 134, 121, 96, 66.

13-Oxo-cleistanth-15-en-18-oic acid [**4**].—Mp 153°; ir ν max (KBr) 3100, 2900, 1732, 1702, 1452, 1393, 1329, 1250, 1220, 1160, 998, 925 cm^{-1}; ^1H nmr (90 MHz) δ 5.90 (1H, m, H-15), 5.15 (1H, dd, *J* = 10 and 1.0 Hz, H-15), 5.08 (1H, dd, *J* = 18 and 1.0 Hz, H-16), 2.90 (1H, dd, *J* = 9 and 4.5 Hz, H-14), 1.12 (s, 19-Me), and 0.7 (s, 20-Me); ms *m/z* $[M]^+$ 304, 208, 193, 148, 139, 135, 95.0498 $[C_6H_7O]^+$ base peak.

$NaBH_4$ REDUCTION OF **4** TO YIELD 13-HYDROXYCLEISTANTH-15-EN-18-OIC ACID [**5**].—$NaBH_4$ was slowly added to a cold MeOH solution of **4** (100 mg), and the mixture stirred for 4 h. The reaction product on work-up afforded **5**, mp 189; ir ν max (KBr) 3450, 2950, 1688, 1463, 1060, 920, 801 cm^{-1}; ^1H nmr (90 MHz) δ 5.90 (1H, m, H-15), 5.23 (1H, dd, *J* = 10 and 1.0 Hz, H-16), 5.11 (1H, dd, *J* = 18 and 1.0 Hz, H-16), 3.56 (2H, m, H-13 and H-14), 1.25 (s, 19-Me), 0.74 (s, 20-Me); ms *m/z* $[M]^+$ 306, 291, 288, 278, 261, 220, 208, 80.

ACETYLATION OF **5** TO YIELD 13-ACETOXYCLEISTANTH-15-EN-18-OIC ACID [**6**].—Compound **5** on treatment with Ac_2O/pyridine yielded **6**, mp 199–200°; ir ν max (KBr) 3500, 2950, 1750, 1708, 1459, 1377, 1247, 1120, 1043, 981, 924 cm^{-1}; ^1H nmr (90 MHz) δ 5.90 (1H, m, H-15), 5.1 (1H, dd, *J* = 10 and 1.0 Hz, H-16), 5.09 (1H, dd, *J* = 18 and 1.0 Hz, H-16), 4.75 (1H, m, H-13), 2.68 (1H, m, H-14), 2.02 (3H, s, *OAc*), 1.36 (s, 19-Me), 0.79 (s, 20-Me); ms *m/z* $[M]^+$ 348, 288, 287, 273, 272, 258, 242, 172, 135, 120.

DEHYDROGENATION OF **1**.—Compound **1** (100 mg) was heated with 10% Pd/C (200 mg) at 300° in N_2 atmosphere for 10 min and repeatedly extracted with $CHCl_3$. After removal of the solvent, the residue on cc (Si gel) gave the following two products.

1,7-Dimethyl-8-ethylphenanthrene.—Mp 108–109°; ir ν max (KBr) 3070, 3020, 2970, 2937, 2875, 1595, 1452, 1378, 1309, 1255, 1210, 1182, 1072, 1040, 828, 805, 771 cm^{-1}; ^1H nmr (400 MHz) δ 8.49 (1H, d, *J* = 8 Hz, H-4), 8.42 (1H, d, *J* = 8.6 Hz, H-5), 7.94 (2H, AB q, A = 7.90, B = 7.99, *J* = 9.4 Hz, H-9 and H-10), 7.44 (1H, t, *J* = 8 Hz, H-3), 7.39 (1H, d, *J* = 8.6 Hz, H-6), 7.34 (1H, d, *J* = 8 Hz, H-2), 3.09 (2H, q, *J* = 7.5 Hz, CH_3CH_2), 2.69 (3H, s, 1-Me), 2.48 (3H, s, 7-Me), 1.23 (3H, t, *J* = 7.5 Hz, CH_3CH_2); ms *m/z* $[M]^+$ 234, 219, 190, 101, 89, 85, 83.

Cleistanth-8,11,13-trien-18-oic acid [**9**].—Mp 141°; ir ν max (KBr) 2900, 1670, 1460, 1440, 1400, 1370, 1320, 1305, 1218, 1200, 1155, 1100, 1060, 1030, 982, 930, 910, 815, 800, 740, 665, 620

cm^{-1}; ^1H nmr (90 MHz) δ 6.98 (1H, d, J=9 Hz, H-11), 6.85 (1H, d, J=9 Hz, H-12), 2.58 (2H, q, J=7 Hz, CH_2CH_3), 2.22 (3H, s, $ArCH_3$), 1.24 (3H, s, 20-Me), 1.21 (3H, s, 19-Me), 1.04 (3H, t, J=7 Hz, CH_2CH_3); ms *m/z* $[M]^+$ 300, 285 (100%), 239, 183, 159, 157, 85, 71, 57, 55.

7-Hydroxycleistanth-13,15-dien-18-oic acid [**10**].—Ir ν max (KBr) 3440, 2950, 2908, 1700, 1660, 1450, 1398, 1239, 1180, 1018, 910, 840 cm^{-1}; ^1H nmr (400 MHz) δ 1.12 ($J_{5,6eq}$=2.4, $J_{5,6ax}$=13.1, H-5), 1.82 (m, H-6ax), 2.13 (m, H-6eq), 3.40 ($J_{7,6eq}$=4.6, $J_{7,6ax}$=11.0, $J_{7,8}$=12.1, H-7), 1.48 (m, H-8), 1.11 (m, H-9), 3.39 ($J_{14,8}$=4.6), $J_{14,15}$=9.8, H-14), 6.06 ($J_{15,14}$=9.2, $J_{15,16}$=9.8, $J_{15,16}$=17.3, H-15), 5.09 (J_{gem}=1.7, $J_{16,15}$=9.8, H-16), 5.20 (J_{gem}=1.7, $J_{16,15}$=17.3, H-16′), 4.73, 4.61 ($J_{17,17'}$=2.3, H-17,17′), 1.24 (Me-19), 0.73 (Me-20); ^{13}C nmr (100.57 MHz) δ 31.2 (C-1), 19.4 (C-2), 39.4 (C-3), 43.6 (C-4), 52.8 (C-5), 32.3 (C-6), 72.0 (C-7), 46.7 (C-8), 48.1 (C-9), 37.3 (C-10), 27.1 (C-11), 37.8 (C-12), 150.8 (C-13), 49.1 (C-14), 136.5 (C-15), 116.6 (C-16), 107.4 (C-17), 182.9 (C-18), 28.9 (C-19), 12.8 (C-20); ms *m/z* 318, 300, 236, 224, 223, 206, 194, 193, 164, 146, 109, 95, 94.

7-Acetoxycleistanth-13,15-dien-18-oic acid [**11**].—Compound **10** (50 mg) on treatment with Ac_2O/pyridine gave **11**, mp 178°, identical in all respects to the natural product, ir ν max (KBr) 3320, 2908, 1724, 1660, 1450, 1380, 1250, 1038, 980, 940 cm^{-1}; ^1H nmr (400 MHz) δ 2.19 (m, H-1), 1.48 (m, H-2ax), 1.71 (m, H-2eq), 1.00 (m, H-3ax), 1.78 (m, H-3eq), 1.19 ($J_{5,6eq}$=2.3, $J_{5,6ax}$=13.2, H-5), 1.75 (m, H-6ax), 2.26 ($J_{6,5}$=2.3, $J_{6,7}$=4.6, J_{gem}=12.5, H-6eq), 4.52 ($J_{7,6eq}$=4.6, $J_{7,6ax}$=11.0, $J_{7,8}$=12.1, H-7), 1.72 (m, H-8), 1.15 (m, H-9), 1.81 (m, H-11ax), 1.88 (m, H-11eq), 1.14 (m, H-12ax), 2.17 (m, H-12eq), 3.16 ($J_{14,8}$=4.6, $J_{14,15}$=9.2, H-14), 6.00 ($J_{15,14}$=9.2, $J_{15,16}$=9.8, $J_{15,16}$=17.3, H-15), 5.05 (J_{gem}=1.7, $J_{16,15}$=9.8, H-16), 4.96 (J_{gem}=1.7, $J_{16,15}$=17.3, H-16′), 4.73, 4.63 ($J_{17,17'}$=2.3, H-17,17′), 1.23 (Me-19), 0.73 (Me-20), 2.07 (CH_3CO); ^{13}C nmr (100.57 MHz) δ 31.0 (C-1), 19.3 (C-2), 39.3 (C-3), 43.7 (C-4), 52.3 (C-5), 28.4 (C-6), 77.0 (C-7), 44.9 (C-8), 46.8 (C-9), 37.2 (C-10), 27.1 (C-11), 37.6 (C-12), 150.7 (C-13), 49.2 (C-14), 136.1 (C-15), 116.8 (C-16), 107.7 (C-17), 183.0 (C-18), 12.7 (C-20), 170.3 (CH_3CO), 21.0 (CH_3CO); ms *m/z* $[M-60]^+$ 300, 285, 205, 146, 133, 109, 94, 92, 91.

7-Oxocleistanth-13,15-dien-18-oic acid [**12**].—Excess Jones's reagent was added to a solution of **10** (100 mg) in Me_2CO and the mixture left overnight. Cc of the product afforded **12**, 60 mg, mp 154°; δ (90 MHz), 3.61 (m, H-14), 5.76 (m, H-15), 5.14 (dd, J_{gem}=1.2, $J_{16,15}$=10.3, H-16 *cis*), 4.98 (dd, J_{gem}=1.2, $J_{16,15}$=17.3, H-16 *trans*), 4.67 (bs, H-17,17′), 1.19 (s, Me-19), 0.84 (s, Me-20); ir ν max (KBr) 3300, 2980, 2910, 1720, 1700, 1660, 1470, 1450, 1300, 1250, 1115, 908 cm^{-1}; ms *m/z* 316, 270, 227, 162 (100%), 154, 149, 144, 121, 119, 109, 107, 105, 93.

METHYLATION OF **10**.—Compound **10** (100 mg) was methylated with CH_2N_2 in Et_2O to give **13** (95 mg), δ (90 MHz) 3.45 (m, H-7 and H-14), 6.02 (m, H-15), 5.28 (dd, J_{gem}=1.2, $J_{16,15}$=10.3, H-16 *cis*), 5.20 (dd, J_{gem}=1.2, $J_{16,15}$=17.3, H-16 *trans*), 4.71, 4.61 (q, J=2.3, H-17,17′), 1.17 (s, Me-19), 0.63 (s, Me-20), 3.63 (s, $COOCH_3$); ir ν max (neat) 3340, 2960, 2908, 1720, 1660, 1450, 1240, 1160, 910, 900 cm^{-1}; ms *m/z* 332, 314, 299, 272, 254, 238, 207, 148, 146, 135, 133, 109, 94, 90.

METHYLATION OF **11**.—Compound **11** (100 mg) on methylation with CH_2N_2 in Et_2O afforded **14** (95 mg); δ (90 MHz) 3.08 (dd, J=4.5, 9.0 Hz, H-14), 4.76 (m, H-7), 5.90 (m, H-15), 4.98 (dd, J_{gem}=1.2, $J_{16,15}$=10.3 Hz, H-16 *cis*), 4.92 (dd, J_{gem}=1.2, $J_{16,15}$=17.3, H-16 *trans*), 4.72, 4.52 (q, J=2.3, H-17,17′), 1.16 (s, Me-19), 0.59 (s, Me-20), 3.55 (s, $COOCH_3$), 2.00 (s, OAc); ir ν max (neat) 3340, 3000, 2904, 1740, 1640, 1380, 1245, 1220, 1165 cm^{-1}; ms *m/z* 374, 332, 314, 254, 206, 146, 145, 134, 94, 92.

WOLFF-KISHNER REDUCTION OF **12** TO YIELD **1**.—Compound **12** (80 mg) was reduced under N_2 in conditions described by Nagata and Itazaki (7) to yield a product which on purification by cc (Si gel) furnished **1** (33 mg) identical in all respects (ir, tlc, nmr) with an authentic sample.

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Received 27 February 1987